COST EMF - MED (Action BM1309):
European network for innovative uses of EMFs in biomedical applications

STSM Report:

Dielectric and magnetic characterization of magnetosomes suspensions (derived from magnetotactic bacteria) in the UHF-SHF frequency ranges for further dosimetric studies

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Abstract:

Chains of magnetite nanoparticles are naturally synthesized by magnetotactic bacteria (MTB) as magnetosomes. There are multiple bio-medical applications of biogenic magnetite. For dosimetric reasons, the electric and magnetic characterization of magnetosomes over a wide frequency band is essential. Two water-based suspensions of magnetosomes from cultured MTB were prepared and analyzed by two methods: a reflection method using an open-ended coaxial probe (0.5-50) GHz and a transmission method using a rectangular waveguide (8.2-12.4) GHz. A very good agreement of measured complex permittivity of a saline solution was obtained with data in literature and convergent results of complex permeability with the one of pure magnetite nanoparticles. Based on these, we report here electromagnetic properties of two magnetosomes suspensions in the UHF/SHF ranges.

A. Purpose of the STSM

By fostering collaboration of our two research groups (Romania and Malta) and by learning/sharing techniques and accessing the infrastructure existent in the Laboratory of Electromagnetics of the University of Malta, we proposed to put into practice a procedure able to provide a reliable characterization of electric and magnetic properties of water-based solutions of magnetosomes in the UHF/SHF ranges. Magnetotactic bacteria *Magnetospirillum gryphiswaldense* (strain DSM 6361) was cultured under microaerobic conditions and harvested when they reached the exponential growth phase, providing magnetosomes which encapsulate nanometric crystals of biogenic magnetite. Similar crystals are present in the human brain and might contribute to less-investigated electromagnetic field effects. Furthermore, biogenic magnetite nanoparticles gained much interest in the last years for many bio-medical applications (magnetic hyperthermia cancer therapy, contrast agents in magnetic resonance imaging, etc.). The bacteria cells were fixed in 1% formalin solutions which were subjected to measurements of complex electric permittivity ε^* and complex magnetic permeability μ^*. These parameters will further enable predictions of SAR values expected from separate E- and H-field components (SAR_E and SAR_H) over wide frequency bands.

B. Work Description

During the whole STSM visit I had the opportunity to observe and to learn how to use various instruments and devices present in the Electromagnetics Laboratory at the Department of Physics, University of Malta. I also had the chance to make some measurements in the anechoic chamber, by using a monopole antenna connected to a generator and amplifier and electric- and magnetic-field probes (SPEAG) for measuring the near-field in air; I have checked the measurement procedure of environmental field level by having access to a NARDA SRM 3006 selective radiation meter; I have also measured the temperature dynamics in a liquid sample with fluoroptic probes by Lumansense Technologies, etc.

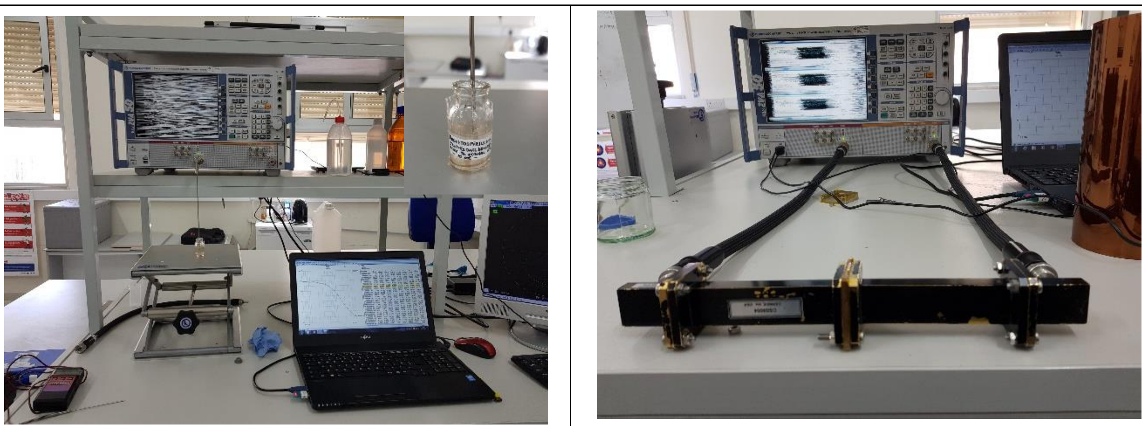
The main instrument I have used during the material-properties measurements (ε* and μ*) was the vector network analyzer (FIG. 1). In order to achieve the main proposed goals of the STSM visit, with the constant help and generous sharing of expertise and knowledge of the team of the Electromagnetics Laboratory, we conducted measurements and experiments as described in the following sections.

The permittivity of a saline solution (0.1 NaCl) and of two magnetosomes suspensions with different concentrations, MAG1 and MAG2, were measured using two different methods, mainly a reflection technique using coaxial probe (FIG. 1a) and a transmission method using rectangular waveguides (FIG 1b). 0.1 NaCl solution was used to validate the two measurement systems. The measurements of 0.1 NaCl obtained using the two measurement systems were compared to previously published data by Peyman et al.

1. The open-ended reflection technique, (0.5-50) GHz

The measurements were conducted in the range 500 MHz-50 GHz by using a slim form probe belonging to the Keysight 85070 kit. The probe was immersed in the liquid as shown in FIG. 1a (up-right corner). The reflection coefficient was measured by the ZVA-50 VNA. From this, the complex permittivity of the material under test was extracted by using an in-house built program based on an equivalent circuit method and also the commercial software Keysight 85071E.

Initially the calibration of the measurement system was made by conducting measurements on three standards, mainly air, short and water. A calibration check was conducted by making measurements on 0.1N NaCl and compared to previously published data. This validated the accuracy of the calibration. Following this stage, measurements on the two different concentrations of magnetosomes samples were conducted. The temperature at which the two magnetosomes samples were investigated were: T1=18.1°C – for MAG1 and T2=18.6°C – for MAG2 (MAG2 had higher magnetosomes concentration than MAG1).



(a) (b)

Figure 1. The two experimental setups used to measure the dielectric properties. (a) depicts the reflection method using an open-ended coaxial probe whilst (b) shows the transmission measurement system using a rectangular waveguide – which allows also measurement of magnetic properties.

2. Rectangular waveguide system measurements, (8.2-12.4) GHz

We used a vector network analyzer (VNA) model ZVA-50 from Rohde&Schwarz and a X-band rectangular waveguide connected to the two ports of the VNA by coaxial cables (FIG. 1b). The scattering parameters (S) were measured using the VNA. Prior to the measurements made on the magnetite samples, a full 2-port calibration of the VNA was done by using thru-reflect-line (TRL) technique. The liquid sample holder consisted of a rectangular waveguide section with the interior dimensions 2.40x1.04x1.04 cm. The sample (magnetosomes suspension) was sealed inside the sample holder by applying Kapton foil on the two lateral faces of the waveguide section. One or two sub-millimeter air bubbles could not be avoided forming during filling/sealing the liquids.

The first liquid to measure was the standard saline solution (0.1% NaCl) at the temperature T1=17.4°C, which was used as a reference. The temperature was measured by a digital thermometer DTM 3000. VNA provided values of the scattering parameters S11, S12, S21 and S22. We used 51 frequency points for the calibrations and conducted measurements in the frequency range of 8.2 to 12.4 GHz. The measurements were repeated three times by refilling the sample holder with the same liquid. At each filling, two different positions of the waveguide segment (holder) were enabled, rotated to fit the waveguide line. A total of six measurements were made for saline. The main problem was the presence of millimetric or submillimetric air bubbles, impossible to eliminate completely, which may have influenced the results in case of all investigated liquids.

By using the 85071E Materials Measurement Software from Keysight/Agilent Technologies, the S-parameters given by VNA were converted to complex permittivity and permeability. We used three models provided by the software for permittivity determination of the saline solution:

- Reflection / Transmission Mu and Epsilon method (called also Nicholson-Ross-Weir / NRW model)
- Reflection / Transmission Epsilon Precision method (called also NIST Precision method)
- Transmission Epsilon Fast method (called also Fast Transmission method).

For measuring the properties of the magnetosomes suspensions samples (they differed by the concentration of magnetite nanoparticles and were denoted here by MAG1 and MAG2), we used the next methods:

- Reflection / Transmission Mu and Epsilon method (called also Nicholson-Ross-Weir / NRW model)
- Reflection / Transmission Mu and Epsilon Polynomial Fit (called also Poly Fit, Bartley-Begley, BB)
- Reflection / Transmission Epsilon Precision (NIST Precision)
- Transmission Epsilon Fast (Fast Transmission)

Only the first two methods allow the determination of μ*. From these two, only NRW model worked properly during experiments, while Poly Fit method provided an error. At such high frequencies, resulted values corresponded rather to non-magnetic behavior of materials.

The measured permittivity values were than compared against the other method, open-ended reflection technique, and against the liquid in which bacteria were preserved, which was distilled water+0.9% NaCl+1% formalin.

3. Exposures in the anechoic room for heating rate determination/incipient dosimetric investigation

An extra experiment, not initially proposed was set-up, in order to check if at frequency f=400 MHz, MAG1 and MAG2 samples heat up when exposed in the near-field of a monopole antenna with an input power of 4 W. The experiment took place in the shielded and anechoic chamber. Two antenna models were used. A monopole omni antenna model Cobham - OA2-0.4V/1604,UK, frequency band 380-400MHz, G=2dBi, vertical polarization, dimensions in mm = 650x36 Ø was fed by a signal generator Lab Bronk Vaunix model LSG-152 (USA), freq. range 250-1500MHz through a power amplifier Mini Circuits ZRL-700+, freq. band 250-700MHz. The maximum input power was set at 4W. The second antenna was also a monopole, but a short one, belonging to a portable transceiver used by security people and police usually in front of the face. A fluoroptic temperature probe model STB with FOT Lab Kit, Lumasense Technologies, was immersed in the magnetosomes solutions to measure local temperature increase, every second (FIG. 2), during exposure. The near field components in the place where the sample of 8ml was positioned, were measured by SPEAG probes: TDS remote unit with field probes optically connected was used, and for E-field we used the probe model TDS E1TDSx, while for H-field measurement we used the model TDS H1TDSx. The probes were connected to the spectrum analyzer FSU from Rohde&Schwarz, freq. range 20Hz-50GHz.

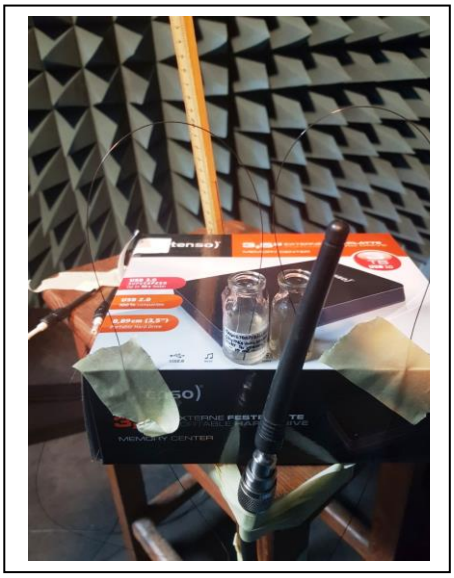


Figure 2. Monopole antenna fed at 4W/440 MHz and magnetosomes suspensions in the near field with inserted optic thermal probes to measure heating during exposure, inside the anechoic chamber.

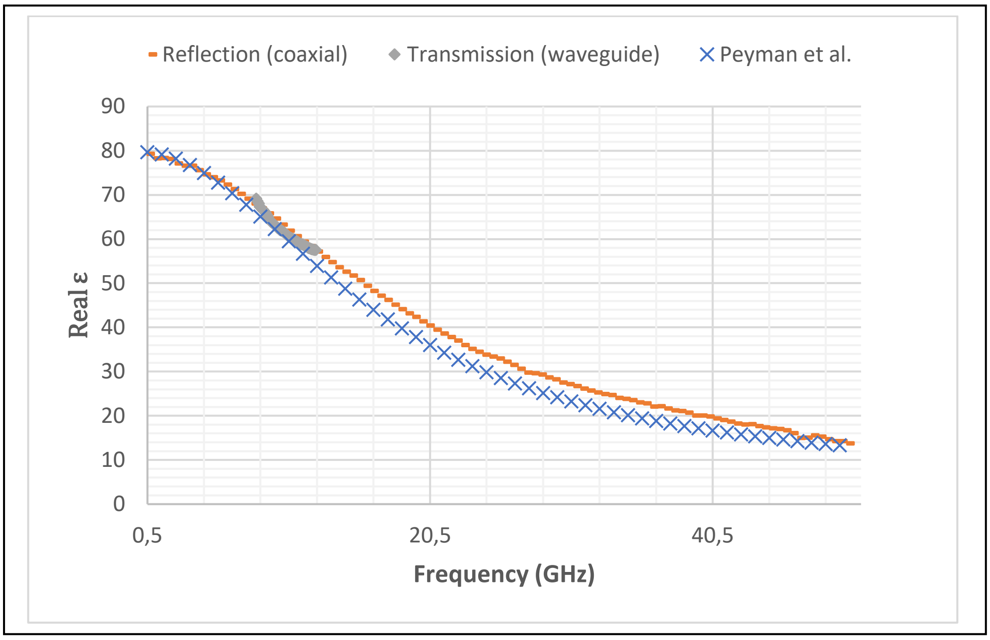
C. Results

FIG. 3 shows the saline dielectric properties as measured by the two methods and compared to results reported by Peyman et al.

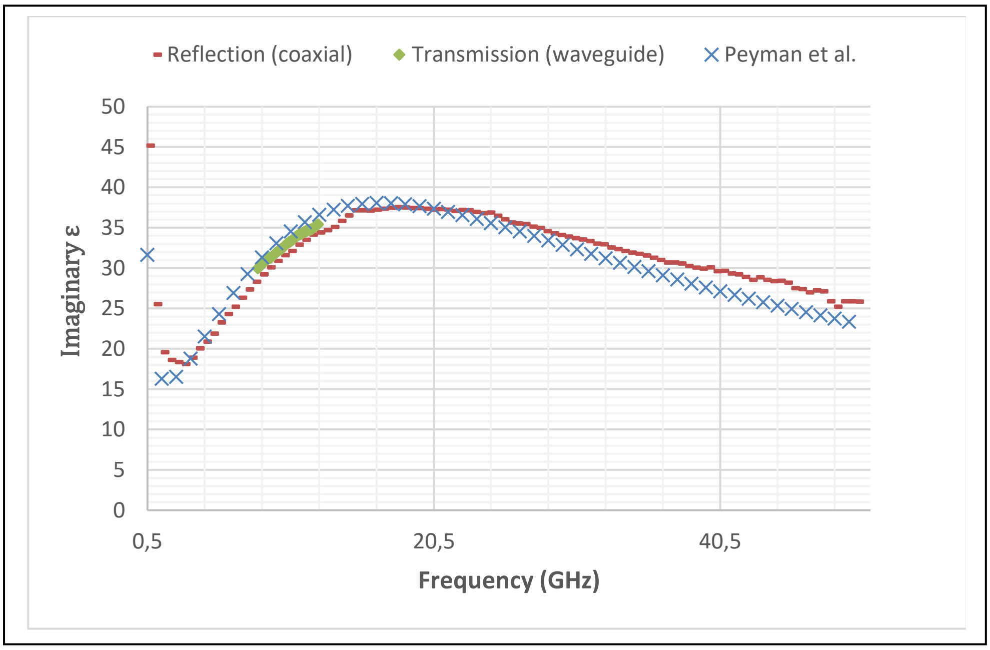
The variation with frequency of ε* and μ* (their real and imaginary parts, separately) for the two magnetosomes suspensions, MAG1 (more diluted) and MAG2 (more concentrated), are presented in FIG.3 - FIG. 5. MAG1 and MAG2 were found to be quite homogeneous as measurements conducted prior mixing the sample were consistent with those after mixing it. This was observed for both the low and high concentration samples. Additionally, no significant difference was noticed between the real and imaginary part of the measured permittivity of both MAG1 and MAG2, even though the concentration of magnetosomes was different. The measured data is presented in FIG. 4, illustrating minimal differences in the imaginary part of permittivity but such differences lie within the experimental uncertainty and therefore cannot be attributed to the difference in concentration. FIG. 4 also presents the data measured using both measurement systems, illustrating good agreement between the two.

By applying NRW model with the rectangular waveguide method, the complex magnetic permeability was measured. FIG. 5 shows the values of real and imaginary permeability of the suspensions MAG1 and MAG 2. We note the very small variation of both components with frequency, between (8.2-12.4) GHz. In the SHF range we observe that magnetite nanoparticles in suspension tend to be diamagnetic. Superparamagnetism, which is the magnetic behavior of the accumulation of several single-domain particles, as the case of magnetosome chains is, means that in absence of an external field the magnetic moment of each particle is oriented randomly and the nanoparticle suspension exhibits no magnetic hysteresis and no static remnant magnetization. We should have expected therefore that a weak external magnetic field to influence the average magnetization of the magnetosomes, like in the case of paramagnetic behavior. However, present measurements indicate rather a diamagnetic behavior of the suspensions.

Since no data are available in literature regarding permeability of biogenic magnetite in SHF, we used for comparison data extracted from Kong et al., 2010, who analyzed synthetic (pure) magnetite nanoparticles over the same frequency range. In FIG. 5 dashed curves indicate permeability of pure magnetite. Interesting to notice is that real part of permeability of pure magnetite and magnetosomes solutions are quite similar, and imaginary part values are not so much different also.

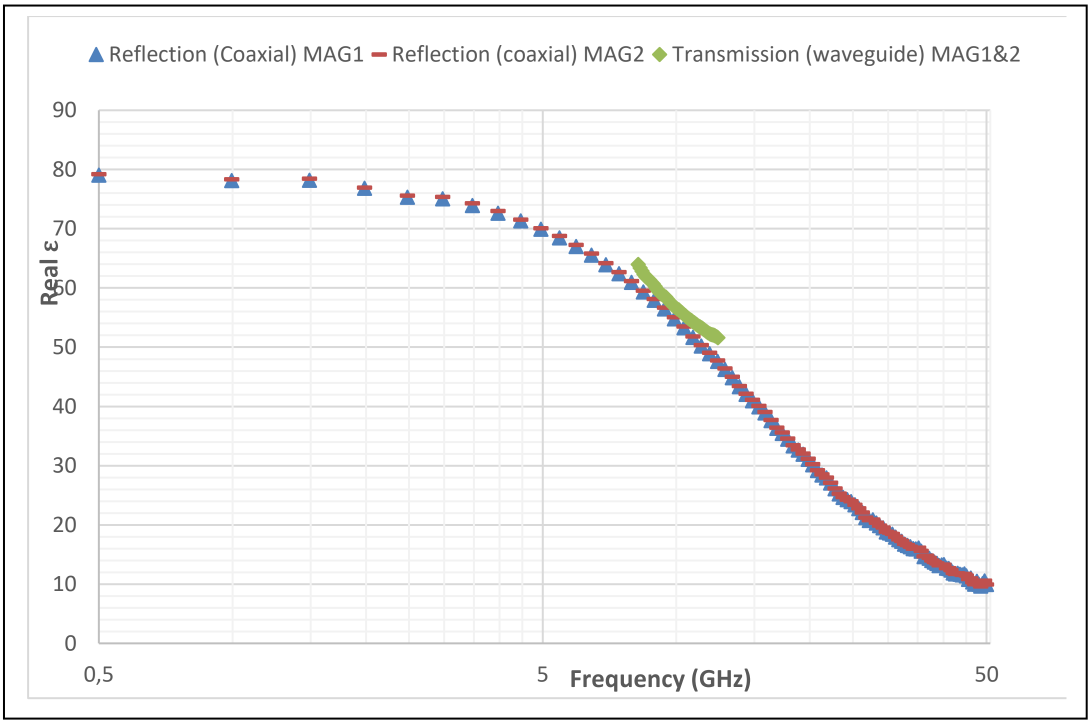


(a)

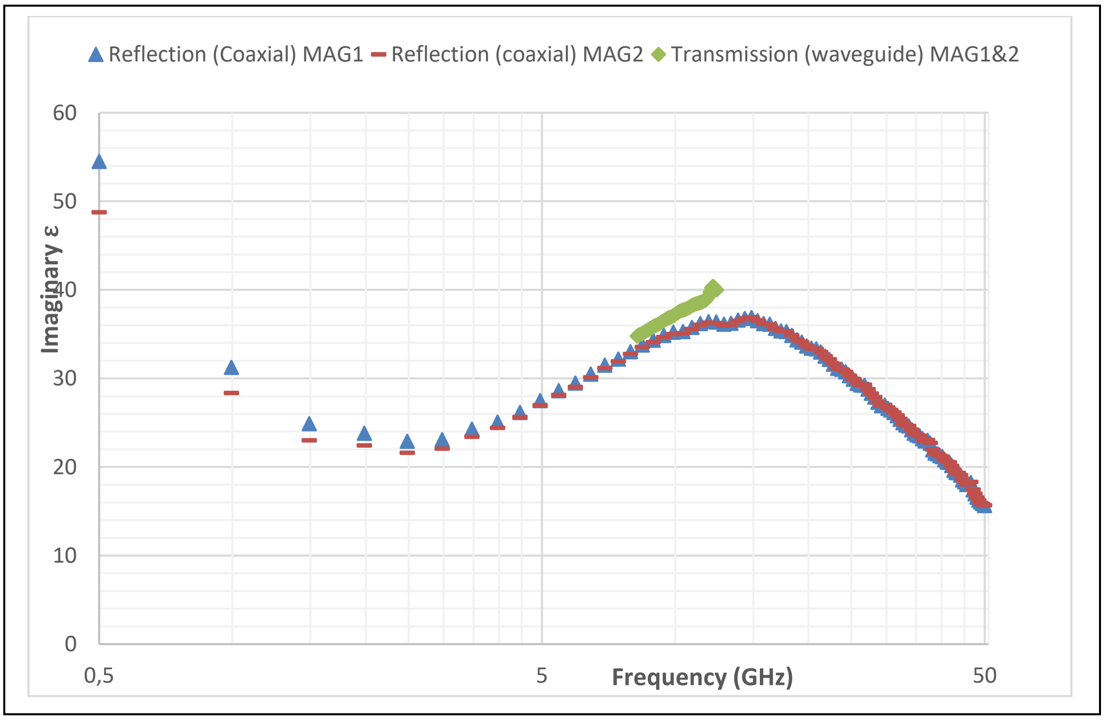


(b)

Figure 3. The real (a) and imaginary (b) part of relative permittivity of 0.1 NaCl at 18°C measured using an open-ended coaxial probe and a rectangular waveguide compared to published data by Peyman et al.



(a)



(b)

Figure 4. The real (a) and imaginary (b) part of relative permittivity as a function of frequency for low (MAG1) and high (MAG2) concentration of magnetosomes samples measured using an open-ended reflection technique together with the mean permittivity for both samples using the transmission method.

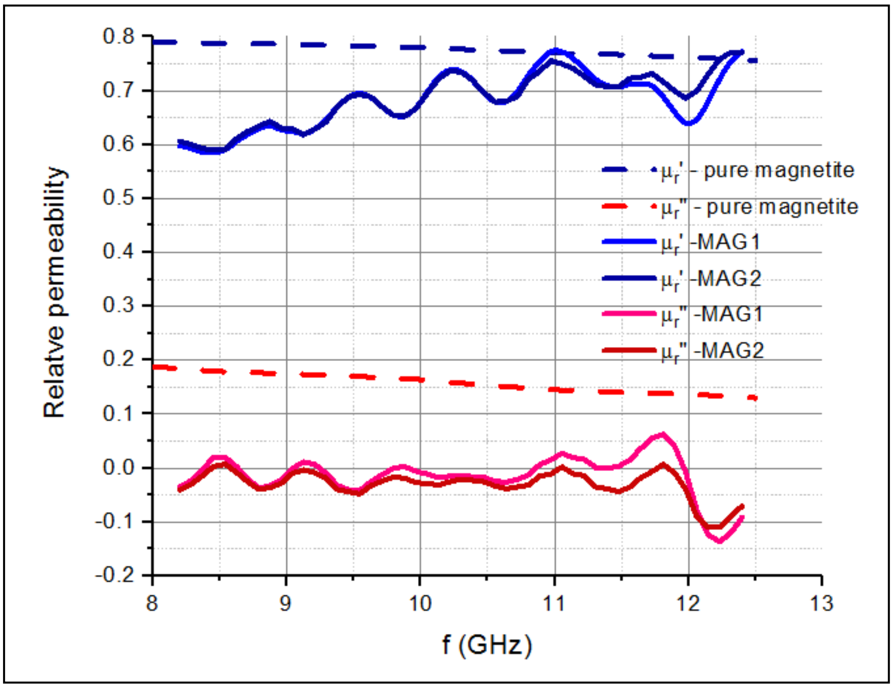


Figure 5. The real and imaginary part of relative permeability of magnetosomes suspensions MAG1 and MAG2 in the SHF range compared to published data on pure magnetite nanoparticles by Kong et al.

References

Peyman A., Gabriel C., Grant E.H., Complex permittivity of sodium chloride solutions at microwave frequencies, Bioelectromagnetics 28(4):264-74, 2007.

Kong I., Ahmad S.H., Abdullah M.H., Hui D., Yusoff A.N., Puryanti D., Magnetic and microwave absorbing properties of magnetite–thermoplastic natural rubber nanocomposites, Journal of Magnetism and Magnetic Materials, 322:3401–3409, 2010.

D. Future collaboration with host institution

- We have planned further characterization of new magnetosomes suspensions (more concentrated) in the lower frequency range, (400-2500) MHz, for further dosimetric studies. Larger quantities of suspensions will be needed, and this will be possible to be obtained in the near future.

- Based on very recent results obtained in Romania with Nuclear Magnetic Resonance relaxation measurements applied to magnetosomes suspensions – demonstrating the potential use of magnetosomal liquids as effective contrast agents in magnetic resonance imaging (MRI), we have together planned to extend our group in order to start MRI tests. The potential collaborators were identified by the Malta group.

- We have discussed and planned exchange of students between our respective institutions utilizing Erasmus+ program.

E. Expected Publications

- JOINT PAPER AT CONFERENCE: At the moment of writing the present report, an abstract was commonly prepared and submitted to **BIOEM 2018 Conference** (June, 2018, Portoroz, Slovenia), enitled: "*The response of magnetotactic bacteria suspensions to radiofrequency in the UHF & SHF ranges: a prospective study*"

- JOINT JOURNAL ARTICLE: A paper is under preparation at present time in collaboration of our groups, and it will be submitted soon to a referee journal indexed by Clarivate Analytics (Master Journal List).

F. Other Comments

During my work in the laboratory, I had the opportunity to discuss and to be helped also by two Ph. D. students: Julian Bonello and Daphne Anne Pollacco. They were both developing their own experimental work on the topics of human tissues dielectric characterization in various conditions and in connection to cancer research. A Greek M.Sc. student following an Erasmus stage was also present at various activities in the laboratory. Regarding my work, I benefited by the competent and tenacious support constantly offered by Dr. Lourdes Farrugia. During the antenna experiment set-up, I was generously helped by Dr. Eman Farhat, while senior researcher Prof. Charles Sammut kindly offered advice during my stay. Interesting discussions I had with an external member of the laboratory, but member of the Faculty, Dr. Malcom Caligari Conti, with whom I learned about electron backscatter diffraction (EBSD) technique which could provide the microstructural-crystallographic characterization of any crystalline or polycrystalline material, like magnetite nanoparticles are. By such discussions, new ideas and collaboration possibilities have opened.

Confirmation by the host institution of the successful execution of the STSM:

We confirm that Simona Miclaus has performed the research work as described above.

Contact Person of Host Institution

Prof. Charles V. Sammut

Signature

Name of researcher

Dr. Simona Miclaus

Signature